Human Leukocyte Antigen-DQB1*03 Alleles Are Associated with Alopecia Areata

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Alopecia areata (AA) is characterized by hair loss in patches (patchy AA), over the entire scalp (AT, totalis), or universally (AU). An autoimmune mechanism has been hypothesized, because the inflammatory infiltrate targeted to the hair follicles includes activated T cells. To investigate whether or not genetic polymorphism of the human leukocyte antigen (HLA) region contributes to disease susceptibility, we used sequence-specific oligonucleotides and amplified genomic DNA to define HLA-DQA1, -DQB1, and -DPB1 alleles in a cohort of 85 white patients. The frequency of DQB1*0301 was significantly increased to 41% in all patients, and to 47% in AT/AU patients relative to controls (27%). Analyzed together, DQB1*03 alleles (DQB1*0301–*0303) were increased to 80% (all patients) and to 92% (AT/AU) (odds ratio = 12.14, p = 0.00003, corrected). This striking association implicates the DQB1*03 alleles in the pathogenesis of AA. DQB1*06 was decreased relative to controls (56%) in all patients (32%, odds ratio = 0.37, p = 0.0045, corrected). An increase was observed in the HLA-DRB1*11(DR5) allele DRB1*1104, which may result from linkage disequilibrium with DQB1 alleles. Sequence comparison among the allele products associated with AA indicates that the DQB1*03 alleles carry a unique proline at position 55 that is not present in alleles that are neutral or negatively associated with the disease. This highly significant association may exert considerable control over immune responsiveness and the initiation or persistence of a T-cell autoimmune response against the hair follicle. ***Key words: autoimmunity/hair/T cells/HLA disease association. J Invest Dermatol 103:758–763, 1994***

Alopecia areata (AA) is a common disease of unknown etiology affecting approximately 1.0% of the population. It ranges in severity from self-limited, small foci of hair loss to persistent, total elimination of all hair [1]. Potentially reversible, it is characterized by either limited patchy hair loss (alopecia areata, patchy AA), loss of all scalp hair (alopecia totalis, AT), or loss of all body hair (alopecia universalis, AU). AA has been suggested to be an autoimmune disease or an immunologically mediated disease because it is associated with other autoimmune disorders [2] and because there is an inflammatory infiltrate of activated T cells surrounding the hair follicles [1,3]. Human leukocyte antigen (HLA) class II expression is seen on follicular keratinocytes in biopsy specimens from AA patients [4–6]. However, this ectopic expression of HLA class II may be secondary to the T-cell infiltrate and indicative of cell injury [7]. The antigen(s) involved and the antigenic specificity of the lymphocytic infiltrate in the AA process are currently unknown.

Like many other autoimmune diseases [8], AA shows significant genetic association with particular alleles of the class II human major histocompatibility complex (MHC). This region on chromosome 6 encodes the highly polymorphic DR, DQ, and DP $\alpha\beta$ heterodimers present on antigen-presenting cells of the immune system [9]. AA has been associated weakly with increased genotypic frequencies of HLA-DR4 and DR5(DRB1*11) in whites [10–12], and with a significant decrease in DRw52a (DRB3*0101) [10]. Odum *et al* [13] also reported HLA-DPB1 associations with AA. Morling *et al* [14] have reported a 4.2-kb Bgl II restriction fragment length polymorphism (RFLP) (associated with HLA-DQB1* 0301) in a small study of 20 Danish AA patients.

Because HLA class II molecules dictate the T-cell repertoire and exert considerable control over immune responsiveness [15], efforts to understand the role of HLA class II genes in disease susceptibility have focused on the detailed characterization of alleles from healthy and diseased individuals using serologic, cellular, and RFLP typing methods. Previous reports of HLA associations with AA have not used the more accurate typing method of hybridization of allele-specific oligonucleotide probes on amplified genomic DNA and have been too small to be of statistical significance. In our efforts to understand the contribution of HLA genes to susceptibility or resistance to patchy AA and AT/AU, we have used this powerful technique to type HLA-DQA1, -DQB1, and -DPB1 alleles in a large white American AA patient population. In addition, by this method we subtyped allelic variants in 38 DR4- and 23 DR5(DRB1*11)-positive AA patients to determine whether the finding of an increased frequency of HLA-DR4 in three separate studies and HLA-DR5 in two separate studies may be due to an association with one of the subtypes of DR4 or DR5 or due to linkage disequilibrium with another HLA-D–region gene.

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Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; HLA, human leukocyte antigen.

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Table I. Oligonucleotide Sequences for Polymerase Chain Reaction Amplification

	Left	Right
HLA-DRB1	5′ GGAGCAGGTTAAACATGAG 3′	5′ CCGCTGCACTGTGAAGCTCT 3′
HLA-DR4	5′ GTTTCTTGGAGCAGGTTAAAC 3′	5′ CCGCTGCACTGTGAAGCTCT 3′
HLA-DR5(11)	5′ CACGTTTCTTGGAGTACTCTAC 3′	5′ CCGCTGCACTGTGAAGCTCT 3′
HLA-DPB1	5′ GAGAGTGGCGCCTCCGCTCAT 3′	5′ GCCGGCCCAAAGCCCTCACTC 3′
HLA-DQA1	5′ ATGGTGTAAACTTGTACCAGT 3′	5′ TTGGTAGCAGCCGGTAGAGTTG 3′
HLA-DQB1	5′ CATGTGCTACTTCACCAACGG 3′	5′ CTGGTAGTTGTGTCTGCACAC 3′

MATERIALS AND METHODS

Subjects After informed consent was obtained, 20 cm^3 of heparinized blood was collected by venipuncture from 200 randomly selected, healthy local white volunteers of predominantly northwest European ancestry. Patients included 85 unrelated white individuals of similar ancestry with AA (patchy AA, n = 47), AT, or AU (AT/AU, n = 38). Controls were screened for a history of AA or other autoimmune diseases. Patients were examined and biopsied, and a diagnosis of patchy AA, AT, or AU was confirmed by a dermatologist.

Preparation of Genomic DNA Genomic DNA from peripheral blood was prepared from buffy coat lymphocytes by proteinase-K/sodium dodecylsulfate digestion, followed by phenol extraction, ethanol precipitation, and storage in Tris–ethylenediaminetetraacetic acid, pH 7.4, at 4°C [16].

Dot-Blot Analysis HLA-DRB1, -DQA1, and -DQB1 loci from genomic DNA were amplified *in vitro* by the polymerase chain reaction technique using TAQ polymerase (Cetus, Emeryville, CA) and a thermocycler (Perkin-Elmer-Cetus, Norwalk, CT) at 94°C × 1 min, 60°C × 2 min, and 72°C × 3 min for 30 cycles. The DPB1 loci were amplified in the same manner at 94°C × 1 min, 61°C × 2 min, and 72°C × 3 min. The amplified DNA was dot blotted onto Zetabind membranes (Cuno, Meridian, CT) and hybridized with ^{32}P-labeled allele-specific or sequence-specific oligonucleotide (SSO) probes at 54°C. Probes were end-labeled with [γ^{32}P]d-ATP using T4 polynucleotide kinase (Promega, Madison, WI) and were added to give no less than 1×10^6 cpm per ml of hybridization fluid. The hybridization buffer was 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, 0.1% sodium dodecyl sulfate, and 5 × Denhardt's solution, containing 100 μg/ml herring sperm DNA and 3.0 M tetramethyl ammonium chloride. The membranes were washed to a stringency of 2 × SSPE in buffer containing tetramethyl ammonium chloride and exposed to Kodak XAR-5 film at −70°C, as described by the Eleventh International HLA Workshop.

Oligonucleotide Primers and Probes Oligonucleotide primers **(Table I)** for polymerase chain reaction amplification of genomic DNA were synthesized using a Beckman desk-top synthesizer and purified using a Milligen/Biosearch oligonucleotide purification column (Millipore, Burlington, MA). SSO probes obtained from the Eleventh International HLA Workshop were used to discriminate further between alleles of HLA-DR4 [DRB1*0401(Dw4), *0402(Dw10), *0403(Dw13.1), *0404(Dw14.1), *0405(Dw15), *0406('KT2'), *0407(Dw13.2), and *0408(Dw14.2)]; between four alleles of HLA-DRB1*11 (DR5) [DRB1*1101, *1102, *1103, and *1104]; between 19 of the alleles that comprise the HLA-DPB1 locus [*0101(DPw1), *0201(DPw2), *0202(DPw2), *0301(DPw3), *0401(DPw4), *0402(DPw4), *0501(DPw5), *0601(DPw6), *0801, *0901(DP'Cp63), *1001, *1101, *1301, *1401, *1501, *1601, *1701, *1801, and *1901]; between nine alleles of DQA1 [*0101, *0102, *0103, *0201, −03 (*03011, *0302), *03012, *0401, *0501, and *0601]; and between 15 alleles that comprise the DQB1 specificities [*0201, *0301, *0302, *0303(*03031, *03032), *0401, *0402, *0501, *0502, *0503 (*05031, *05032), *0504, *0601, *0602, *0603, *0604, and *0605]. In this report, allelic designations are used when appropriate, with the previous designations for HLA-DR, HLA-DQ, or HLA-DP specificities given in parentheses.

Statistical Analysis Comparison of HLA specificities and allelic frequencies in patients with AA or AT/AU versus normal race-matched controls was performed using the χ^2 analysis of 2 × 2 tables and Yate's correction with the EPI-INFO statistical program. If five or fewer persons were present per group, Fisher's exact two-tailed test was used. The p values were corrected for multiple comparisons, i.e., HLA-DQA1, p value × nine alleles tested, unless otherwise noted. If an association had been reported previously, the p value was not corrected. Based on previous observations, the *a priori* hypothesis included that HLA-DR4, DRB1*11 (DR5), DQ7 (DQB1*0301), and DPw4 alleles might be associated positively with AA. Odds ratios (OR) or relative risks and exact confidence levels were determined from the 2 × 2 tables.

RESULTS

Increased Frequency of HLA-DQB1*03 Alleles in AA Patients DQB1 alleles were defined by amplification of the DQB1

Table II. HLA-DQB1 Frequencies in AA Patients and White Controls

	AA/AT/AU (n = 85)			Patchy AA (n = 47)			AT/AU (n = 38)			Normal (n = 200)
HLA-DQB1	Frequency % (n)	p	OR	Frequency % (n)	p	OR	Frequency % (n)	p	OR	Frequency % (n)
*0201	25% (21)			21% (10)			29% (11)			36% (72)
DQB1*03	80% (68)	0.00003	4.16	70% (33)	>0.05	2.45	92% (35)	0.00003	12.14	49% (98)
*0301	41% (35)	0.026[a]	1.89	36% (17)			47% (18)	0.021[a]	2.43	27% (54)
*0302	25% (21)			21% (10)			29% (11)			17% (34)
*0303	14% (12)	>0.05	3.12	13% (6)			16% (6)	>0.05	3.56	5% (10)
*0401	0% (0)			0% (0)			0% (0)			0% (0)
*0402	7% (6)			9% (4)			5% (2)			7% (14)
*0501	25% (21)			32% (15)			16% (6)			29% (58)
*0502	12% (10)	0.015	6.53	11% (5)	>0.05	5.83	13% (5)	>0.05	7.42	2% (4)
*0503	2% (2)			2% (1)			3% (1)			4% (8)
*0504	0% (0)			0% (0)			0% (0)			0% (0)
DQB1*06	32% (27)	0.004	0.37	36% (17)	>0.05	0.45	26% (10)	0.015	0.28	56% (112)
*0601	5% (4)			4% (2)			5% (2)			3% (5)
*0602	21% (18)			23% (11)			18% (7)			26% (52)
*0603	2% (2)	0.03	.012	2% (1)			3% (1)			17% (33)
*0604	4% (3)			6% (3)			0%			11% (22)
*0605	0% (0)			0% (0)			0%			0

[a] p uncorrected, based on previously documented association (Morling *et al* [14]). For all others, the p value is corrected by multiplying by the number of alleles tested (15 alleles).

Table III. HLA-DQA1 Frequencies in White AA Patients[a]

	AA/AT/AU (n = 47)	Patchy AA (n = 23)	AT/AU (n = 24)	Normal (n = 200)
HLA-DQA1	Frequency % (n)	Frequency % (n)	Frequency % (n)	Frequency % (n)
*0101	30% (14)	39% (9)	21% (5)	33% (66)
*0102	38% (18)	39% (9)	38% (9)	37% (73)
*0103	4% (2)	9% (2)	0% (0)	18% (35)
*0201	21% (10)	9% (2)	33% (8)	20% (40)
*03	40% (19)[b]	30% (7)	50% (12)[c]	25% (50)
*0301	0% (0)	0% (0)	0% (0)	6% (12)
*0401	13% (6)	17% (4)	8% (2)	6% (12)
*0501	38% (18)	38% (9)	38% (9)	36% (71)
*0601	2% (1)	0% (0)	4% (1)	1% (2)

[a] p values are corrected (nine alleles tested).
[b] $p > 0.05$, OR = 2.04.
[c] $p > 0.05$, OR = 3.0.

locus in 85 white patients, followed by hybridization with SSO probes **(Table II)**. A significantly increased frequency of DQB1*0301 was observed in this group of patients (41% compared to 27% in controls, $p = 0.026$, uncorrected). In addition, the frequency of the combined DQB1*03 alleles (*0301–*0303) was significantly increased (80% compared to 49% in controls, $p = 0.00003$, corrected). In the subgroup of severe AT/AU patients, the frequency of DQB*0301 was increased to 47%, compared to 27% in normal white controls ($p = 0.021$, uncorrected). Most striking, an increased frequency of the combined DQB1*03 alleles (92% compared to 49% in controls, $p = 0.00003$, corrected) was significantly associated with the AT/AU phenotype. An increase in the frequency of the DQB1*0502 allele was significantly associated with disease when all patients were combined (12% compared to 2% in controls, $p = 0.015$, corrected).

We also observed alleles that were reduced in AA patients. A significant decrease in the frequency of the HLA-DQB1*0603 allele was observed in AA/AT/AU patients (2% compared to 17% in controls, $p = 0.03$, corrected). In addition, a decrease in the combined DQB1*06(*0601–*0605) alleles was significantly associated with the severe AT/AU phenotype (26% compared to 56% in controls, $p = 0.015$, corrected). The decreased frequency of the DQB1*06 alleles was also significant for the total patient group (32% versus 56% in controls, $p = 0.004$, corrected).

Analysis of the HLA-DQA1 alleles from the 47 white patients examined in this study identified an insignificant increase in the frequency of the DQA1*03 allele and a decrease in the frequency of the DQA1*0103 allele in all patients **(Table III)**. The frequency of the DQA1*0501 allele in this patient cohort was not increased, as reported previously by Morling *et al* [14] using RFLP typing.

To address this question further, we analyzed the role of the combined presence of DQB1*0301 and DQA1*0501 in the patient group versus controls **(Table IV)**. When patients and normal white controls who possessed the DQA1*0501 allele were analyzed for the combined presence of DQB1*0301 and DQA1*0501, the OR was significantly increased in the patients (OR = 8.06, $p = 0.007$, uncorrected). One hundred percent of AT/AU patients who possessed the DQA1*0501 allele also had the DQB1*0301 allele, and 80% of the AT/AU patients who carried the DQB1*0301 allele also had the DQA1*0501 allele (OR = 2.89, $p = 0.046$). When analyzed together, 33% of all patients typed for HLA-DQB1 and -DQA1 alleles carried the DQB*0301/DQA*0501 combination, compared to 17% of controls (OR = 2.44, $p = 0.027$).

HLA-DPB1 Alleles Are Not Associated With AA A report finding increased numbers of patients with HLA-DPw4 in AA [13] led us to perform HLA-DPB1 typing of 84 AA patients and 62 normal white controls. In this study, we were able to subtype the HLA-DPB1*04 (DPw4) into DPB1*0401 and *0402 alleles. The other DPB1 epitopes were defined according to the Eleventh International HLA Workshop. Amplified genomic DNA hybridized with SSO probes was used to define the HLA-DPB1 alleles **(Table V)**. The phenotypic frequencies of the DPB1*0401 and *0402 alleles in all patients were 58% and 25%, respectively, and are similar to the control frequencies of 69% and 27%. The frequency of the DPB1*0101 allele was decreased, but not significantly, in both patchy AA and AT/AU patients versus controls (8% versus 18%, respectively). All other HLA-DPB1 alleles were similar to control frequencies, suggesting that in this group of patients, HLA-DPB1 alleles are not associated with AA.

HLA-DRB1 Alleles Associated with AA Eighty-eight white AA patients had been HLA-DR–typed previously using RFLP techniques [10]. Sixty-one percent of the patients had either HLA-DR4 or DRB1*11 (DR5), compared with 40% of white controls. To define alleles that may be involved in disease susceptibility, we performed subtyping of AA patients with either HLA-DR4 or DRB1*11 (DR5) specificities. Group-specific primers were used to amplify the DRB1*11 (DR5) region of genomic DNA, which then was hybridized with seven SSO probes to define four DRB1*11 (DR5) alleles: HLA-DRB1*1101, *1102, *1103, and *1104. Six of the seven DRB1*11 (DR5)–positive patchy AA patients were of the DRB1*1104 subtype (86%), versus 27% of the DRB1*11 (DR5) white controls (OR = 16.25, $p = 0.034$, corrected). When all patchy AA and AT/AU patients were analyzed together, DRB1*1104 was found in 11 of 20 patients tested (55%), compared with the frequency of DRB1*1104 in normal HLA-DRB1*11(DR5)–positive controls (27%) **(Table VI)**.

HLA-DR4 subtyping by generic or group-specific amplification of genomic DNA and hybridization with allele-specific oligonucleotide or SSO probes was used to define the HLA-DR4 alleles in 38 HLA-DR4–positive AA patients. Allelic frequencies were not significantly different from HLA-DR4 control frequencies (data not shown). Therefore, these results do not define a significant association between any subtype of HLA-DR4 and AA.

Table IV. Frequency (%) and OR for the Allelic Combination of HLA-DQB1*0301 and HLA-DQA1*0501 in AA[a]

	AA/AT/AU			Patchy AA			AT/AU			Normal
Patients	Frequency	p	OR (RR[b])	Frequency	p	OR (RR)	Frequency	p	OR (RR)	Frequency
All	33% (14/43)	0.027	2.44 (1.74)	29% (6/21)	>0.05		36% (8/22)	>0.05		17% (33/200)
DQB*0301–positive	82% (14/17)	>0.05		86% (6/7)	>0.05		80% (8/10)	0.046	2.89 (2.20)	61% (33/54)
DQA1*0501–positive	88% (14/16)	0.007	8.06 (1.88)	75% (6/8)	>0.05		100% (8/8)	0.005	ND[c] (2.20)	46% (33/71)

[a] p values are uncorrected based on previously documented association (Morling *et al* [14]).
[b] RR, relative risk.
[c] ND, not done.

Table V. Frequency of HLA-DPB1 Alleles Defined by Hybridization of Amplified DNA With SSO Probes in Healthy White and AA Patients

HLA-DPB1 Alleles[a]	Patchy AA (n = 36)	AT/AU (n = 48)	All (n = 84)	Controls (n = 62)
*0101	8% (3)	8% (4)	8% (7)	18% (11)
*0201	22% (8)	31% (15)	27% (23)	23% (14)
*0202	0% (0)	10% (5)	6% (5)	2% (1)
*0301	11% (4)	15% (7)	13% (11)	18% (11)
*0401	69% (25)	50% (24)	58% (49)	69% (43)
*0402	4% (5)	33% (16)	25% (21)	27%(17)
*0501	6% (2)	8% (4)	7% (6)	5% (3)
*0601	8% (3)	2% (1)	5% (4)	3% (2)
*0901	8% (3)	0% (0)	4% (3)	3% (2)
*1001	11% (4)	0% (0)	5% (4)	2% (1)
*1101	3% (1)	13% (6)	8% (7)	8% (5)
*1401	8% (3)	2% (1)	5% (4)	5% (3)
*1501	6% (2)	0% (0)	2% (2)	2% (1)

[a] The phenotypic and genotypic frequencies of the remaining DPB1 alleles, *0801, *1301, *1601, *1701, *1801, and *1901, were less than or equal to 5% in all groups tested (patchy AA, AT, AA/AT, and white controls).

DISCUSSION

This study strongly implicates DQB1*03 alleles in the pathogenesis of AA. Although weak HLA class II associations, including DR4 and DRB1*11(DR5), have been reported previously for AA [10–12,17], serologic or RFLP HLA typing methods were used. DNA RFLPs associated with DQB1*0301 and DQA1*0501 were reported in 20 Danish AA patients. However, this was a very small study limited to one population [14]. It is important to note that the investigators did not analyze the severe AT/AU patients separately from the less severe patchy AA patients. In addition, RFLP typing cannot unequivocally identify critical polymorphic HLA alleles, which can be identified by the methods employed in our study. The goal of this study was to determine whether genetic polymorphisms of the HLA region contribute to disease susceptibility or resistance in AA. Using allele-specific or sequence-specific oligonucleotide probes, we provide a molecular analysis of HLA-DQA1, -DQB1, DRB1, and -DPB1 alleles that may be important in the pathogenesis of AA and define previously unreported HLA allelic associations with AA.

The expression of HLA-DQB1 alleles in AA patients is not random and shows a highly significant association of DQB1*03 alleles. Ninety-two percent of AT/AU patients carried one of the closely related allelic variants, HLA-DQB1*0301, *0302, and *0303. The difference between these three alleles is limited to polymorphic residues at positions 13, 26, 45, and 57 of the DQB1 gene product. Sequence comparison shows that only the amino acid at position 55 of the DQ beta chain is unique in the sequences of the DQB1*03 alleles, compared with all other DQB1 alleles. All of the DQB1*03 alleles positively associated with AA/AT/AU have proline at position 55. Arginine or leucine is present at position 55 in all alleles that are neutral or negatively associated with disease **(Table VII)**.

Elucidation of the structure and function of major histocompatibility molecules has provided a molecular basis for HLA associations with disease [18–20]. Critical polymorphic amino acid residues confer differences in peptide binding and ultimately in antigen presentation to T cells, and may contribute to susceptibility to autoimmune diseases [21,22]. Recent resolution of the three-dimensional structure of the class II HLA-DR1 molecule has allowed correlation of these polymorphic sites with functional regions of the molecule [20]. Based on the class II HLA-DR1 structure, the polymorphic residue at position 55 of the DQ beta chain does not appear to be a direct peptide contact site. However, the unique proline at position 55 of the DQ beta chain that is associated with AA/AT/AU may have subtle effects on other residues involved in peptide binding, which would change the conformation of the peptide-binding groove.

Human HLA-DR and -DQ molecules are expressed on the cell surface as $\alpha\beta$ heterodimers. In crystal forms of HLA-DR1, a dimer of class II $\alpha\beta$ heterodimers has been observed. Although it is possible that dimer formation is a crystallization artifact, it has been hypothesized to be involved in intracellular signaling events [20]. Residues whose side chains are in the dimer interface include positions 52 and 55 of the beta chain. Using the class II HLA-DR1 structure as a model for HLA-DQB1*03 alleles, allelic products with side chains of proline at position β 55 would be incapable of forming a salt bridge at the dimer interface. Therefore, in addition to a possible effect on the peptide-binding groove, polymorphism at position 55 may affect the stability or structure of any dimer formation of $\alpha\beta$ heterodimers.

Genotyping for HLA-DQB1 genes in these patients also showed an increase in the frequency of the DQB1*0502 allele, which may result in an increased risk to develop AA or persistent disease. The DQB1*0502 allele overrepresented in the patient population carries a unique serine residue at position 57 of the DQ beta chain. Because it is predicted to reside on the floor of the peptide-binding site, this may affect peptide class II interactions. The only other allelic product of the HLA-DQB1 locus that possesses a serine residue at position 57, DQB1*0504, was not identified in the patient or control population.

In this study, we were unable to confirm a proposed association of HLA-DQA1*0501 with disease [14]. However, the frequency of the combined presence of DQA1*0501 and DQB1*0301 was significantly increased over controls, confirming this previously published report. These results may suggest that an additive or synergistic effect exists between DQB1*0301 and DQA1*0501 alleles. Family studies will determine whether DQA1*0501 and DQB1*0301 reside on the same chromosome in these patients. In addition, we could not confirm an association between the serologically defined DPB1*04(w4) and AA as reported for a Danish population [13]. Subtyping of the DPB1*04(w4) alleles, DPB1*0401 and *0402, which was not done in the previous study, showed no increase in either allele in these AA patients. Similar results were obtained when we analyzed the combined frequency of DPB1*0401 and *0402 alleles.

Although associations between HLA-DQ and -DR with AA have been reported, the results have been controversial. Our previous studies used conventional RFLP techniques and showed an increase in the frequencies of HLA-DQw8(w3) alleles (found on DR4 haplotypes) in all patients [10]. HLA-DQw7(w3) was not significantly increased; however, the number of heterozygotes with DQw7(w3)

Table VI. Frequency (%) of Alleles HLA-DRB1*11 (DR5) in AA

HLA-DRB*11(DR5) Alleles	Patchy AA (n = 7)	AT/AU (n = 13)	All (n = 20)	White Controls[a] (n = 26)
DRB1*1101	14% (1)	54% (7)	40% (8)	50% (13)
DRB1*1102	0% (0)	8% (1)	5% (1)	15% (4)
DRB1*1103	0% (0)	0% (0)	0% (0)	8% (2)
DRB1*1104	86% (6)[b]	38% (5)	55% (1)	27% (7)

[a] One homozygous control.
[b] $p = 0.008$, uncorrected two-tailed Fisher's exact test; $p = 0.032$ (× four alleles tested) versus white controls. OR = 16.25.

Table VII. Conserved Region of Sequence Polymorphism in HLA-DQB1 Alleles Expressed in AA Patients

	AA Position							
DQB1 Allele	50	51	52	53	54	55	56	57
Disease associated								
301	V	T	P	L	G	**P**	P	D
302	V	T	P	L	G	**P**	P	A
303	V	T	P	L	G	**P**	P	D
Neutral or negative disease associated								
501	V	T	P	Q	G	**R**	P	V
503	V	T	P	Q	G	**R**	P	D
504	V	T	P	Q	G	**R**	P	S
601	V	T	P	Q	G	**R**	P	D
602	V	T	P	Q	G	**R**	P	D
603	V	T	P	Q	G	**R**	P	D
604	V	T	P	Q	G	**R**	P	V
605	V	T	P	Q	G	**R**	P	V
201	V	T	L	L	G	**L**	P	A
401	V	T	P	L	G	**R**	L	D
402	V	T	P	L	G	**R**	L	D

and DQw8(w3) was increased relative to controls. In several reports using serologic or RFLP techniques, the frequency of DRB1*11(DR5) was increased. However, these studies used small cohorts and could not distinguish among allelic variants of the DRB1*11 (DR5) family [10–12,17]. The data reported here suggest that the increased frequency observed for DRB1*11(DR5) may result from an increased number of patients with the HLA-DRB1*11 (DR5) allele, DRB1*1104. This association with DRB1*1104 may result from linkage disequilibrium with DQB1*03 alleles. It is unlikely that the increased frequency observed in our patient population could be due to differences in the ethnicity of our patients versus controls. The 25 white DRB1*11 (DR5)–positive controls were split equally among northwestern and eastern European ancestry, whereas the AA patients were predominantly of northwestern European descent. Because the DRB1*1104 allele is found more frequently in eastern Europeans, the opposite result would have been predicted based on ethnicity alone [23].

DQB1*03 alleles associated with persistent disease (AT/AU) have a relative risk (OR) of 12.14. The OR associated with DRB1*1104 for patchy AA is 16.25. Associations of HLA class II and other autoimmune diseases have been quantified in terms of relative risk [24]. For example, the relative risk associated with HLA-DR4 for insulin-dependent diabetes mellitus is 4–6; for HLA-DR3 and -DR7 associated with Celiac disease, reported relative risks are 8–12; for HLA-DR4, w6 associated with pemphigus vulgaris, the relative risk has been reported to be as high as 24. Compared to previously reported relative risks for HLA-associated diseases, the relative risks for DQB1*03 alleles associated with severe AT/AU and for DRB1*1104 associated with patchy AA are profound.

Our results suggest the possibility that more than one HLA allele can influence susceptibility to AA, or possibly result in persistence of the disease once triggered. It is not surprising that all patients with alopecia areata do not have the same allele. The presence of disease in individuals of differing HLA types could be explained by gene conversion-like events, which shuffle the hypervariable segments among different alleles [25–28]. This model is supported by studies of rheumatoid arthritis, associated with HLA-DR4 (DRB1*0401, *0404/8, *0405) and DR1(*0101) [29,30], and of insulin-dependent diabetes mellitus, associated with DR3 and DR4 [31]. Our results describing three closely related, disease-linked allelic variants, HLA-DQB1*0301, *0302, and *0303, which share a unique amino acid residue at position 55, also support this model of gene conversion.

Alternatively, there could be different susceptibility epitopes, perhaps on different loci or on different chromosomes, that contribute to disease pathogenesis. This model would explain the HLA association of DQB1*03 alleles and the DQB1*0502 allele with disease, as well as reported IgG heavy-chain associations with AA [32].

The fact that certain major histocompatibility complex polymorphisms predispose an individual to aberrant immune responses leading to a disease state may provide insights about the underlying pathogenesis of AA. Further analysis of the role of these residue(s) in the immune response of AA may provide information about specific pathogenic antigens that might be bound by the disease-linked HLA polymorphisms. Much progress has been made in identifying class II sequences that confer susceptibility or resistance to many autoimmune diseases. However, our understanding of how HLA molecules function in disease development remains unclear. Perhaps, in addition to the results presented here, studies examining autoreactive T cells from AA patients, candidate autoantigens, or virally induced immune reactions in AA will provide important clues about the role of HLA molecules in the pathogenesis and persistence of patchy AA, AT, and AU.

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